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**Project Identity**      **NAG 2-756**

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**Project Title**      **CELL KINETIC AND HISTOMORPHOMETRIC  
ANALYSIS OF MICROGRAVITATIONAL  
OSTEOPENIA: PARE.03B**

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## **PROJECT DESCRIPTION :**

**NAG 2-756: CELL KINETIC AND  
HISTOMORPHOMETRIC ANALYSIS OF  
MICROGRAVITATIONAL OSTEOPENIA: PARE.03B**

### **A. Hypotheses**

#### **Flight Studies**

Osteoblast histogenesis is blocked during a 9 day spaceflight but rapidly recovers within hours to days after return to earth.

### **B. Specific Aims**

1. **Assess incidence of bone formation sites and rates of apposition at 4-6, 24 and 72 h following flight. Extrapolate back to estimate the actual flight effect.**
2. **Determine if the microgravitational block in osteoblast histogenesis is systemic or localized to specific types of bones by assessing:**
  - a) **Periodontal ligament (PDL) - nonweightbearing**
  - b) **Mandibular condyle - non-weightbearing, antigravity postured**
  - c) **Tibial metaphysis - weightbearing**
  - d) **Lumbar vertebrae - continuous remodeling secondary spongiosa**
3. **Determine the time course for recovery of osteoblast production by assessing the cell kinetics of osteoblast histogenesis 4-6, 24 and 72 h following return to earth.**

## **SUMMARY OF EXPERIMENTAL FINDINGS**

### **I. Pre-Flight Experiment To Compare 5-Bromo-2'-Deoxyuridine (BrdU) Immunohistochemistry And <sup>3</sup>H-Thymidine (3HT) Autoradiography**

#### **Summary Of Data**

Previous methods of identifying cells undergoing DNA synthesis (S-phase) utilized <sup>3</sup>H-thymidine (3HT) autoradiography. 5-Bromo-2'-deoxyuridine (BrdU) immunohistochemistry is a nonradioactive alternative method. This experiment compared the two methods using the nuclear volume model for osteoblast histogenesis in two different embedding media. Twenty Sprague-Dawley rats were used, with half receiving 3HT (1  $\mu$ Ci/g) and the other half BrdU (50  $\mu$ g/g). Condyles were embedded (one side in paraffin, the other in plastic) and S-phase nuclei were identified using either autoradiography or immunohistochemistry. The fractional distribution of preosteoblast cell types and the percentage of labeled cells (within each cell fraction and label index) were calculated and expressed as mean  $\pm$  standard error. Chi-Square analysis showed only a minor difference in the fractional distribution of cell types (Fig. 1). However, there were significant differences ( $p < 0.05$ ) by ANOVA, in the nuclear labeling of specific cell types. With the exception of the less-differentiated A+A' cells, more BrdU label was consistently detected in paraffin than in plastic-embedded sections. In general, more nuclei were labeled with <sup>3</sup>H-thymidine than with BrdU in both types of embedding media (Fig 2.). Labeling index data (labeled cells/total cells sampled  $\times$  100) indicated that BrdU in paraffin, but not plastic gave the same results as 3HT in either embedding method. Thus, we conclude that the two labeling methods do not yield the same results

for the nuclear volume model and that embedding media is an important factor when using BrdU. As a result of this work, 3HT was chosen for used in the PARE.03 flight experiments.

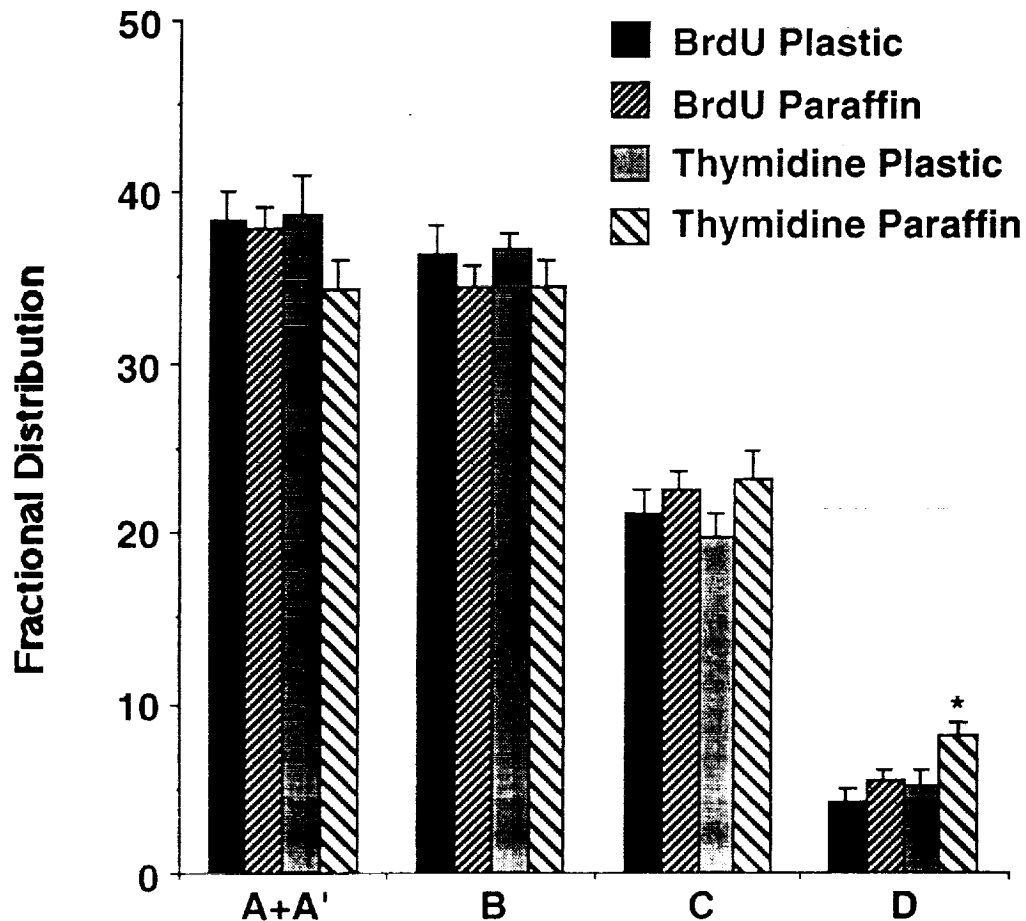


Figure 1 Comparison of cell fractional distributions for tissues treated with either BrdU or  $^3\text{H}$ -thymidine and embedded in either plastic or paraffin. No differences were observed except for the D cell compartment where the number of cells in the thymidine treated, paraffin embedded group was slightly higher ( $p < 0.05$ ) than the other groups. This effect is likely a chance event since all other categories were remarkably similar.

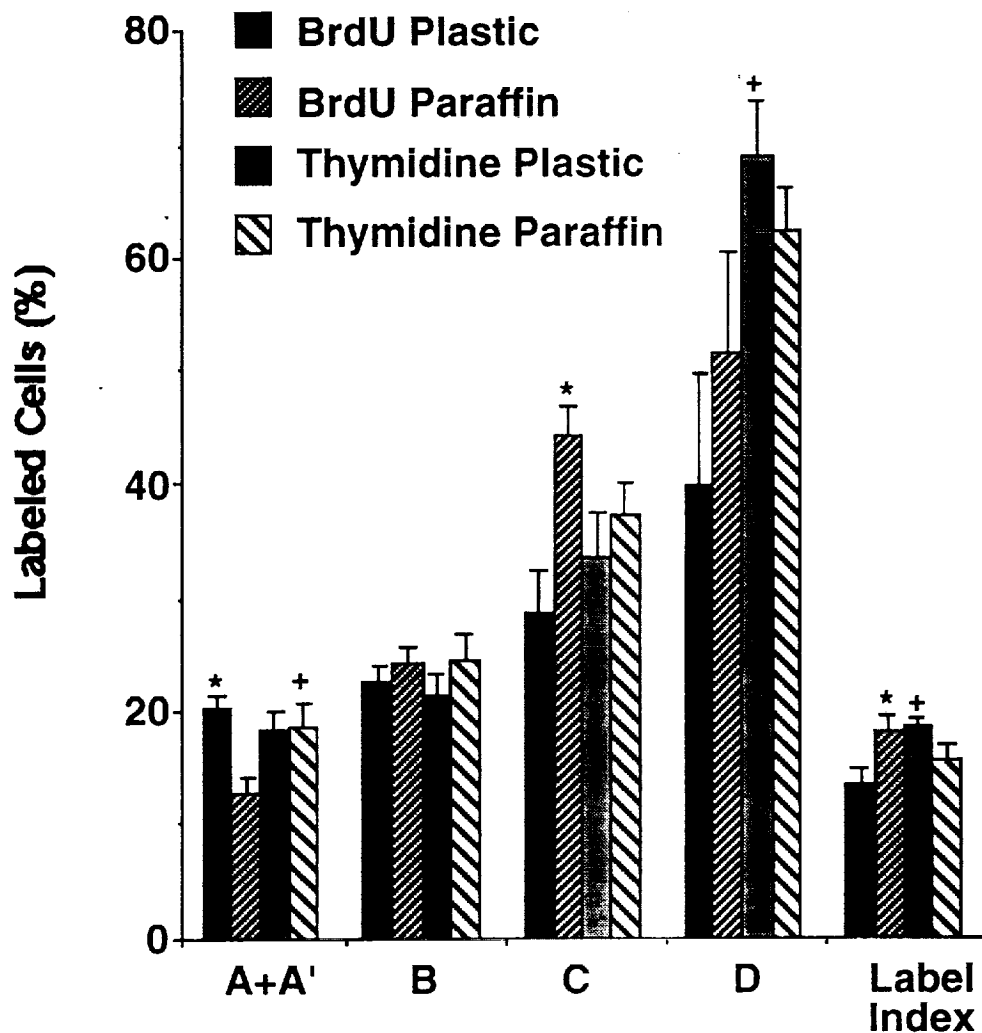


Figure 2

Graph of percent labeling of S-phase cells within nuclear volume compartments, and label index sample (not measured for nuclear volume). Significant differences appear between \*embedding media in the BrdU-labeled A, C, and Label index samples ( $p < 0.01$ ) and +labeling methods in the paraffin-embedded A cells ( $p < 0.05$ ), and in the plastic-embedded D cell compartments ( $p < 0.05$ ), and Labeling index sample ( $p < 0.01$ ).

## II. Recovery of Osteoblast Histogenesis in Rat Periodontal Ligament Following a 9-day Spaceflight (PARE.03)

### Summary of Data

The influence of a ~9 day space flight and ~4, 24 and 72 hour recovery period at 1 g was assessed in fibroblast-like osteoblast precursor cells from rat maxillary molar periodontal ligament (PDL). Using a nuclear morphometric assay, PDL from flight (F) and flight-control animals housed in Animal Enclosure Modules were analyzed in ~4  $\mu\text{m}$  hematoxylin and eosin stained sections for the relative number of preosteoblasts (C+D cells), less-differentiated progenitor cells (A+A' cells) and nonosteogenic (B) cells (where A+A'=40-79  $\mu\text{m}^3$ ; B=80-119  $\mu\text{m}^3$ ; C+D>120  $\mu\text{m}^3$ ). No differences were observed in the PDL width in any of the groups (Fig. 3). In addition, no differences in the fractional distribution of PDL cells were observed among the three recovery periods in control animals (Fig. 4). The control groups from the recovery time periods were combined for comparison to tissues from flight animals. As shown in Figure 5, four hours after landing, A+A' cells from F animals were elevated above control levels (F:  $26.7 \pm 3.1$  vs. Control:  $16.7 \pm 2.0$ ;  $p < 0.05$ ; data expressed as % of total cells; mean  $\pm$  SEM for  $n = 6$  animals). Statistically, these less-differentiated progenitor cells had returned to control levels at 24 h and 72 h postflight (F-24 h:  $23.5 \pm 2.3$ ; F-72 h:  $15.8 \pm 2.8$ , respectively;  $n = 4-5$  animals). Conversely, preosteoblasts (C+D cells) tended to be depressed at both 4 and 24 h following flight (F 4 h:  $43.2 \pm 3.6$ ; F- 24 h:  $42.8 \pm 2.4$  vs. Control:  $53.8 \pm 3.1$ ), but were back to control levels by 72 h (F-72 h:  $58.2 \pm 3.0$ ). No differences were observed in the fractional distribution of nonosteogenic (B) cells (F-4 h:  $26.8 \pm 1.8$ ; F-24 h:  $31.6 \pm 2.3$ ; F-72 h:  $24.8 \pm 1.5$  vs. Control:  $26.7 \pm 2.4$ ). These data support previous findings and suggest a microgravity-induced block in osteoblast precursor cell differentiation that rapidly recovers following return to a 1 g environment.

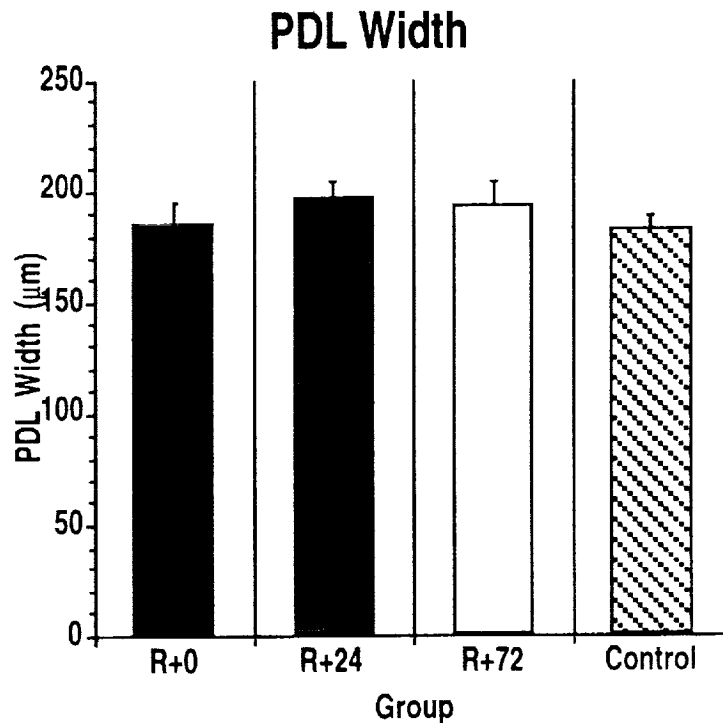


Figure 3 PDL Width in  $\mu\text{m}$ . No differences were observed in any of the flight and control groups.

Figure 2

## Control Comparison

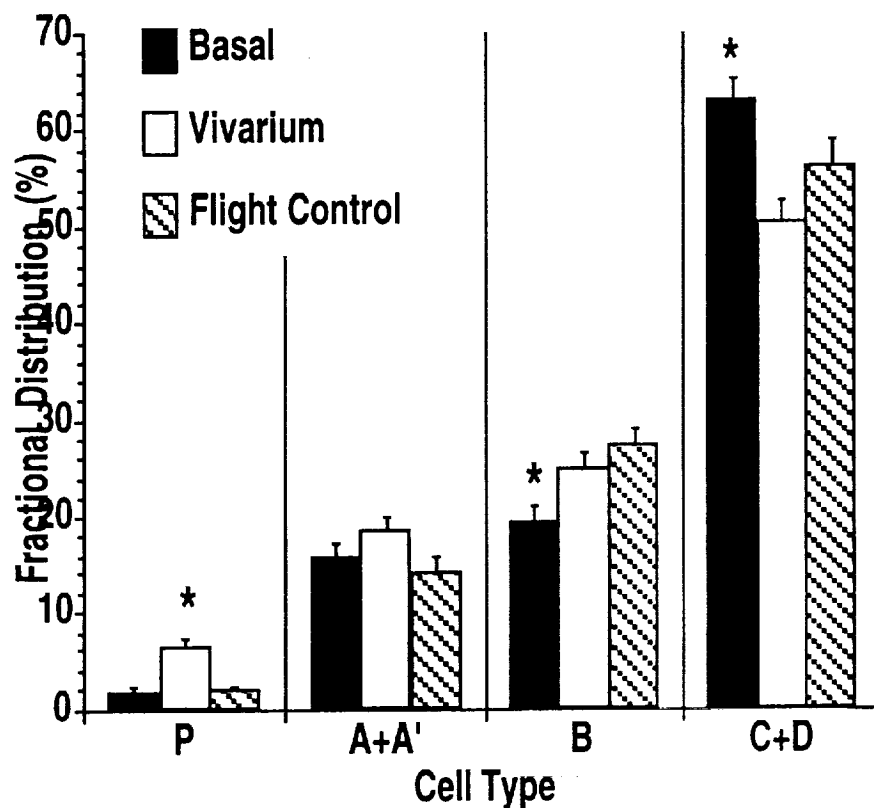


Figure 4  
Comparison of Cell fractional distribution in non-flight control groups. \*indicates a difference at  $p < 0.05$  from the Basal group.

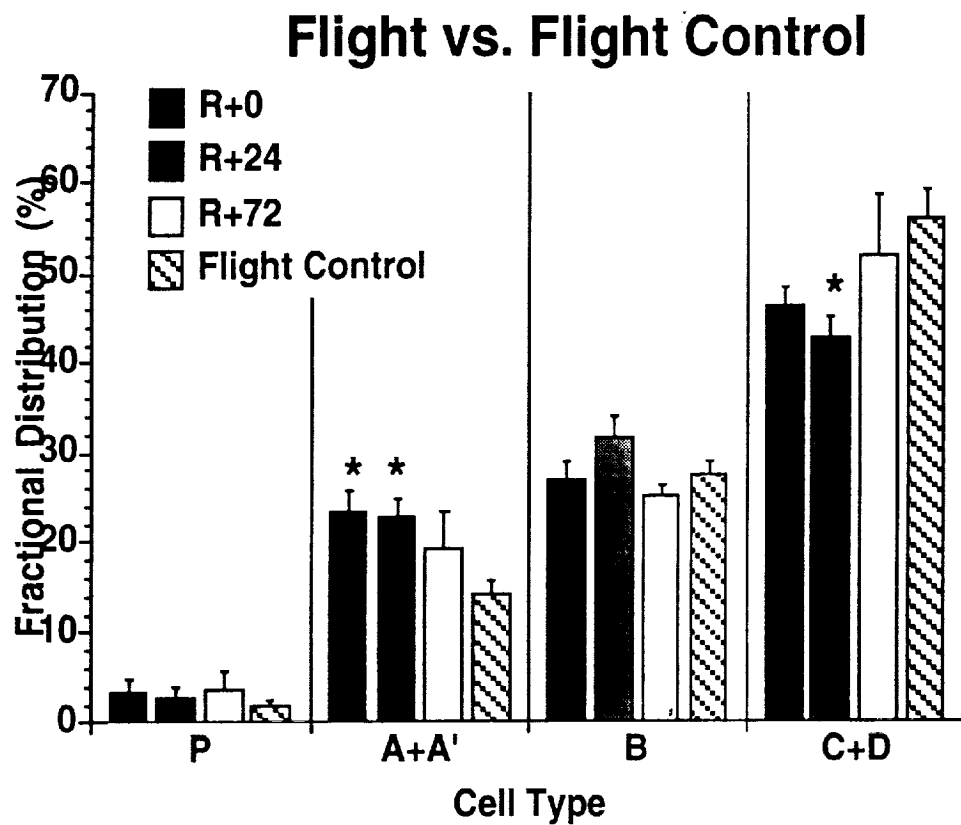


Figure 5  
Cell fractional distribution for flight and flight control groups at each of the time periods studied. \* indicates  $p < 0.05$  for Flight vs. Flight Control.



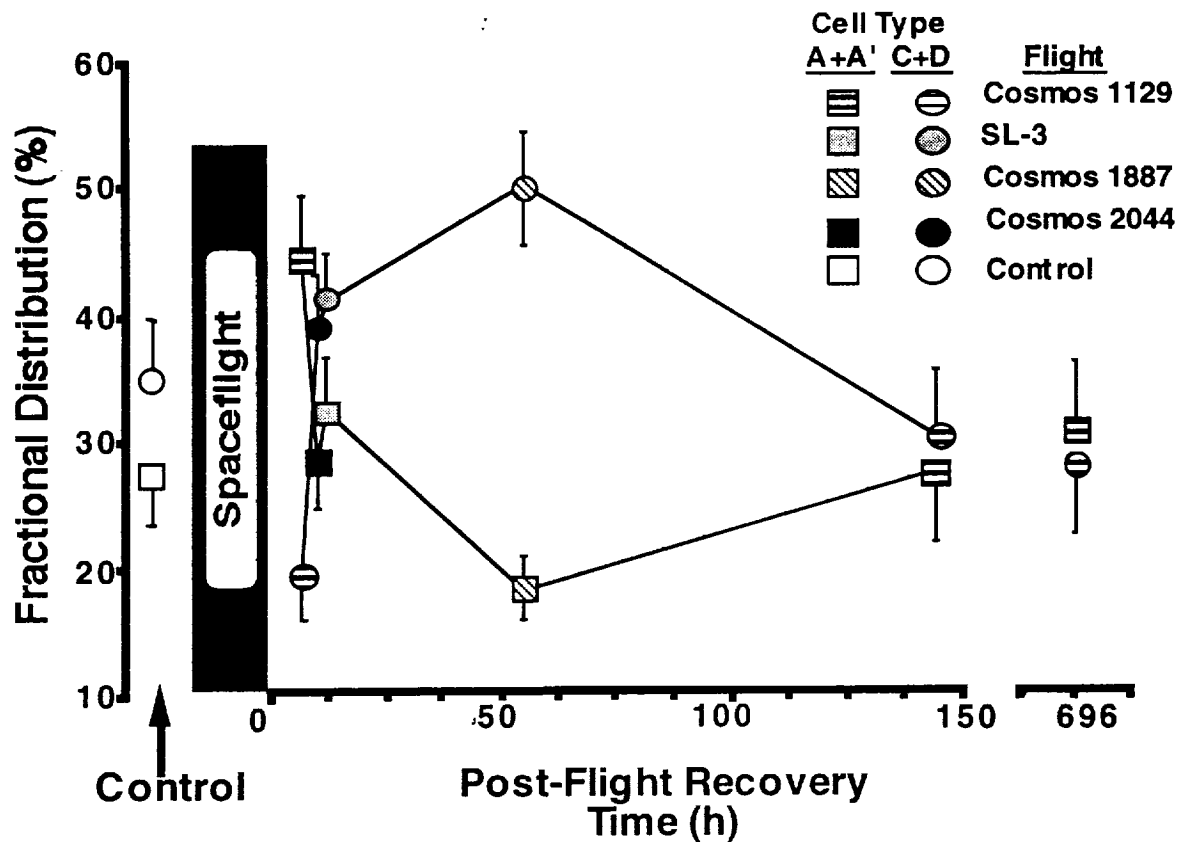


Figure 6 Summary of recovery data from spaceflight experiments. These data indicate that there is an initial postflight inhibition of preosteoblast production ( $\downarrow$  C+D cells) and concomitant increase in early progenitor cells ( $\uparrow$  A+A' cells). Following return to earth there is a transient return through control levels at  $\sim 10$  h postflight. Finally there appears to be a strong osteogenic recovery response ( $\uparrow$  C+D and  $\downarrow$  A+A' cells) at  $\sim 55$  hours postflight and a return to control levels by at least 6 d post flight. The data from the PARE.03 experiments confirms the early block in osteoblast histogenesis and indicates that the return to preflight levels may occur as early as 72 hours following flight.

### III. Analysis of Mandibular Condyles from PARE.03 Flight Experiment

#### Summary of Data

##### Gross Morphometric Analysis

##### 1. Morphometric Analysis of PARE.03 Mandibles

Post-flight morphometric measurements of the mandibular condyle were expected to show whether microgravity resulted in growth-related defects at secondary cartilage areas. Analysis of mandible morphometry revealed no differences in flight vs. control groups. (See Figures 7 and 8)

2. These data represent a pattern identical to that found during analysis of long bone specimens from PARE.03A by Holton et al.

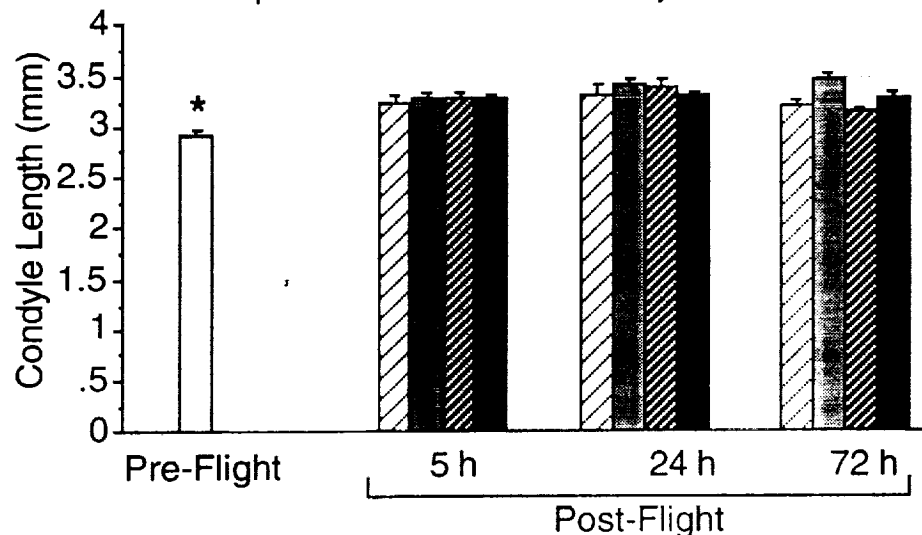


Figure 7

Measurement of condyle length indicates that animals were growing normally and that exposure to the microgravity environment of spaceflight did not inhibit this growth. \*indicates a significant difference from all other groups at  $p < 0.05$ .

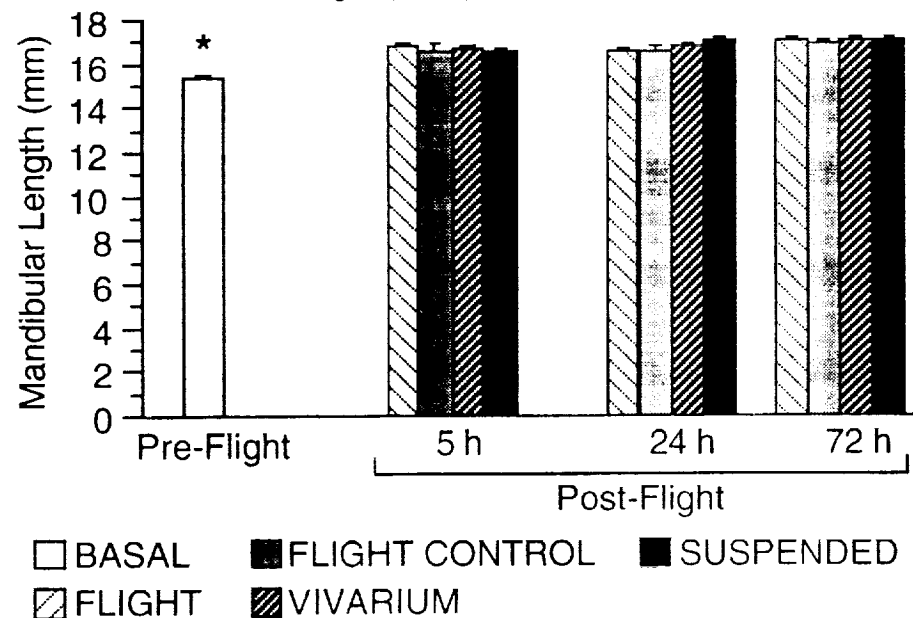
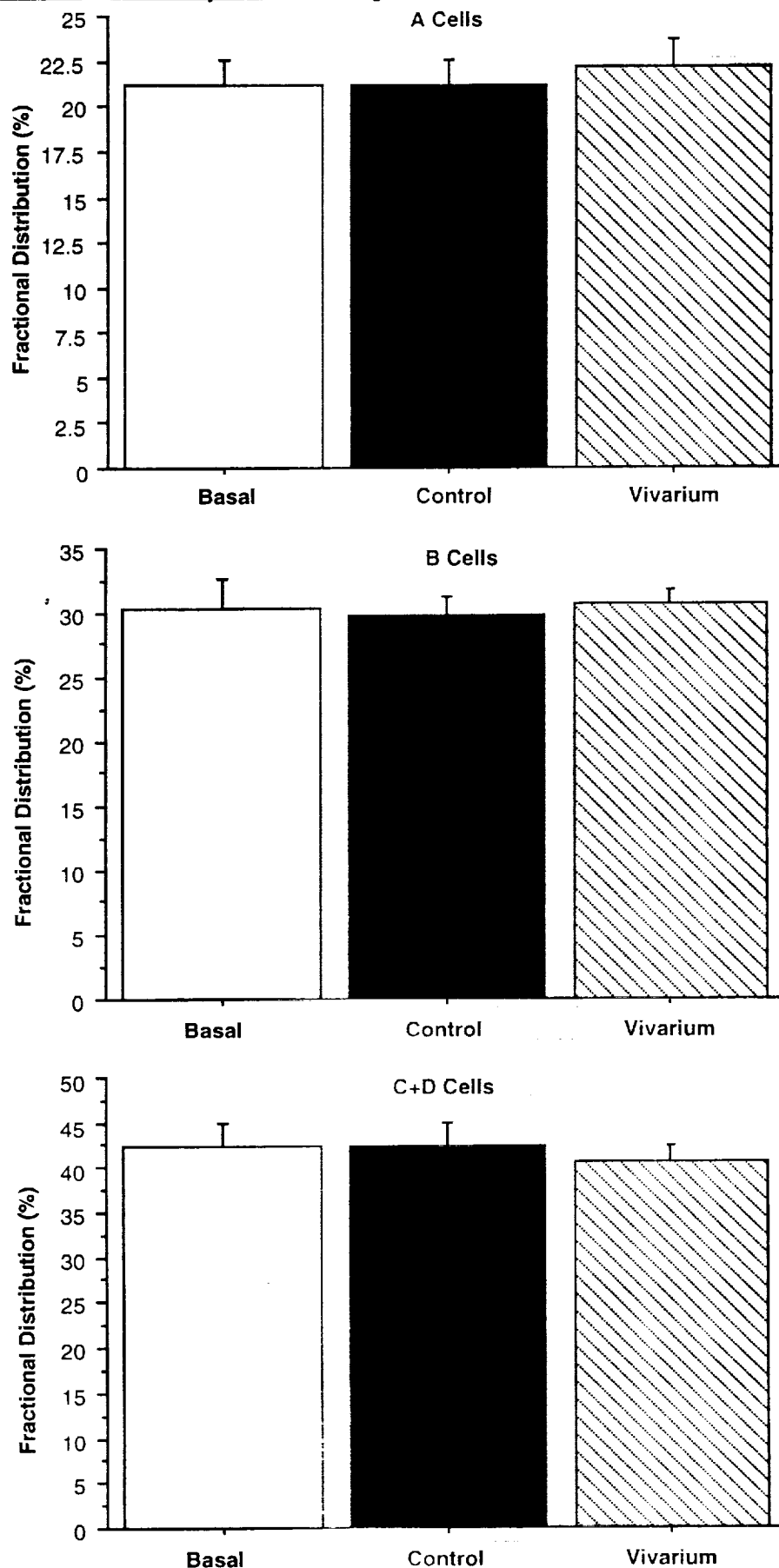


Figure 8.

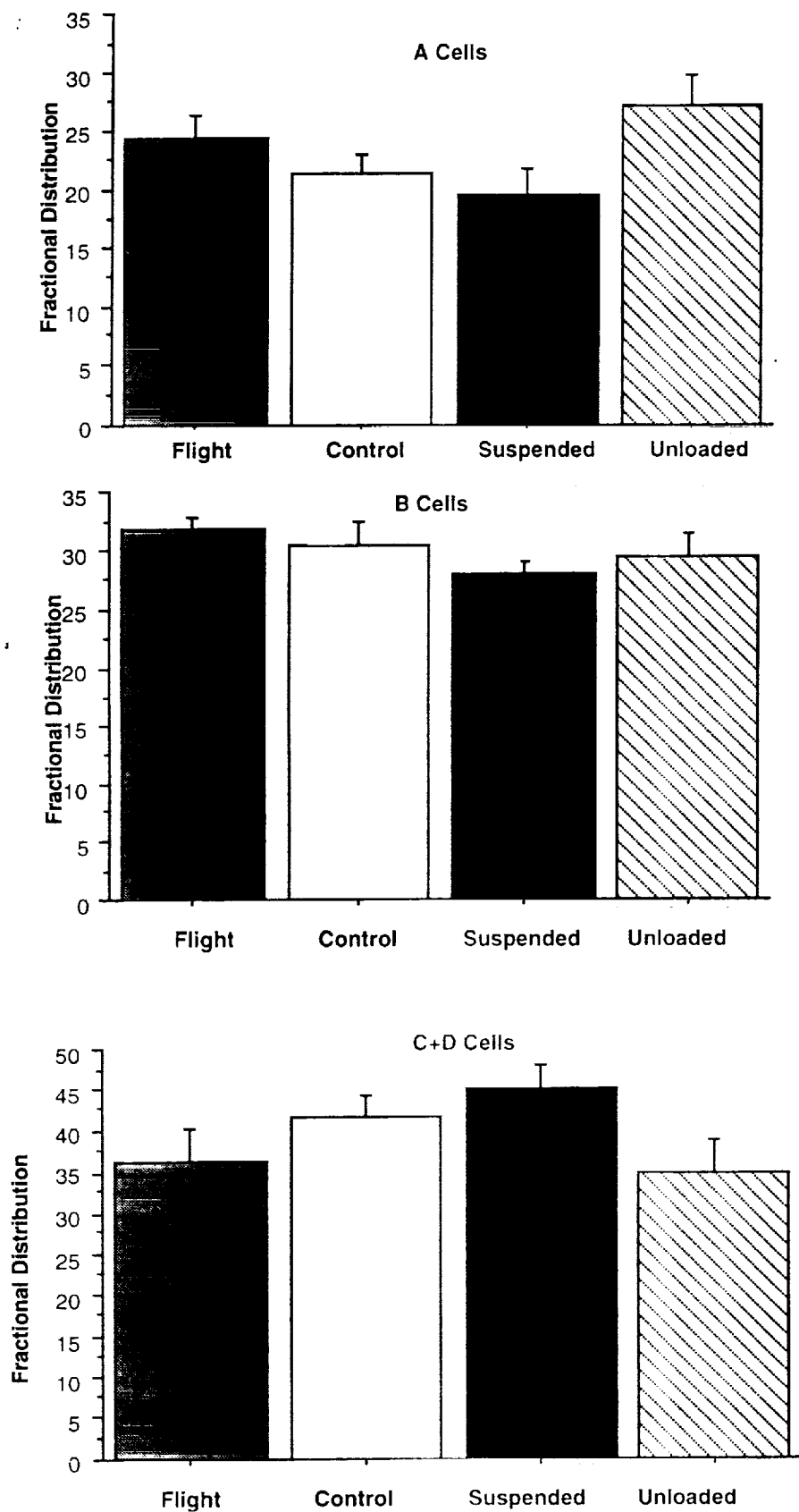
Measurement of overall mandibular body length likewise indicates that animals were growing normally and that exposure to the microgravity environment of spaceflight did not inhibit this growth. \*indicates a significant difference from all other groups at  $p < 0.05$ .

# Cell Kinetic Analysis of Osteogenic Precursors in the Mandibular Condyle



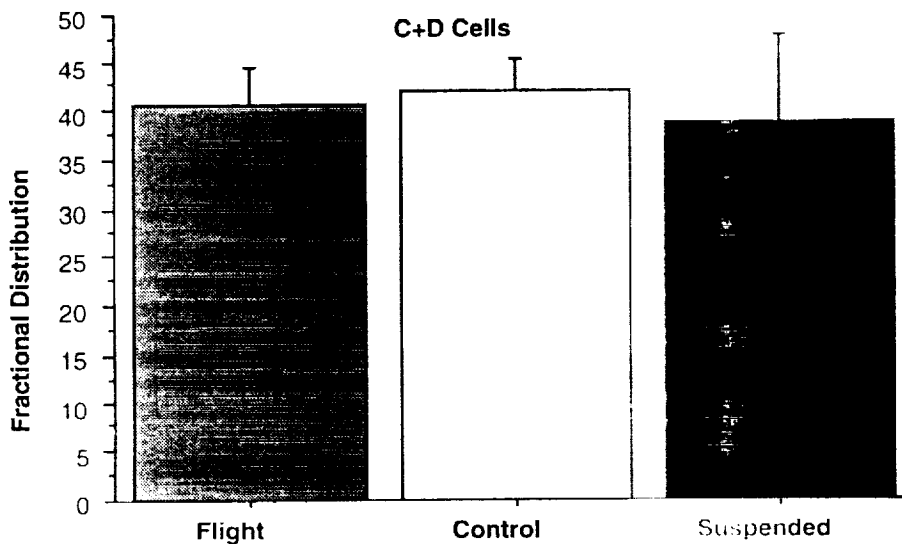
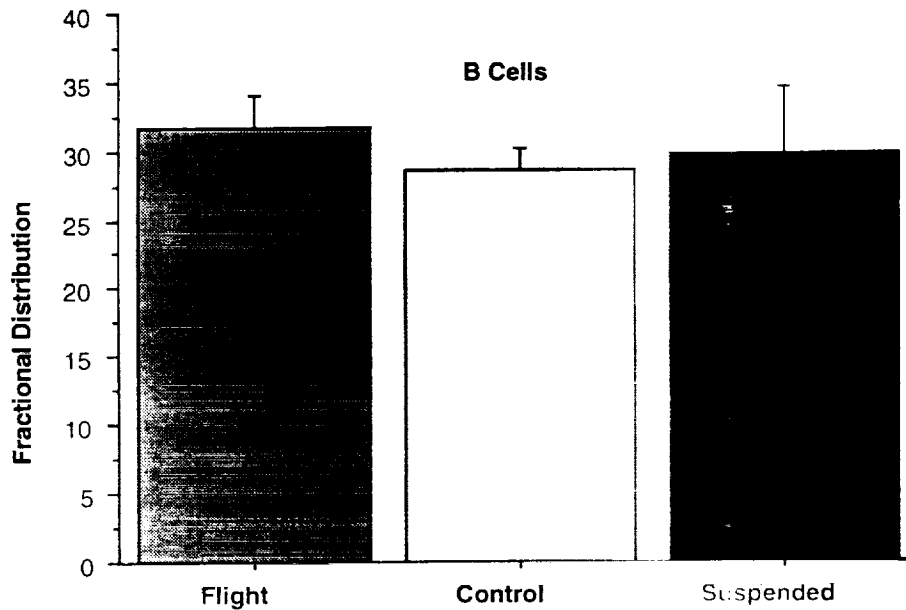
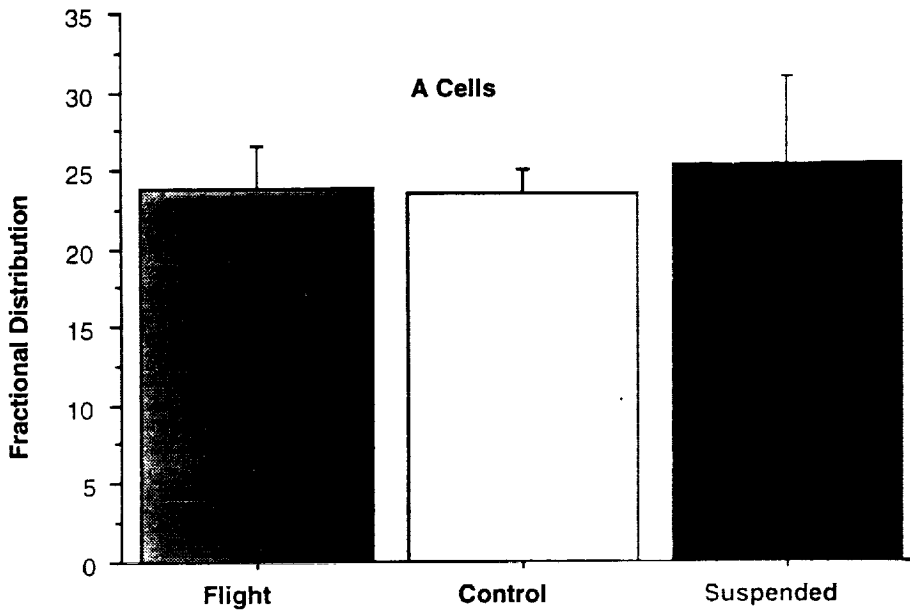
**FIGURE 9:**

Comparison between the three control groups (basal, flight control and vivarium) for the A, B and C+D cell fraction. Bars indicate standard error. No differences were found in the fractional distribution of cell types between the control groups. Because flight control and vivarium groups are the same age and because they were represented in all timepoints, the data for these two groups were combined for comparison to flight groups.



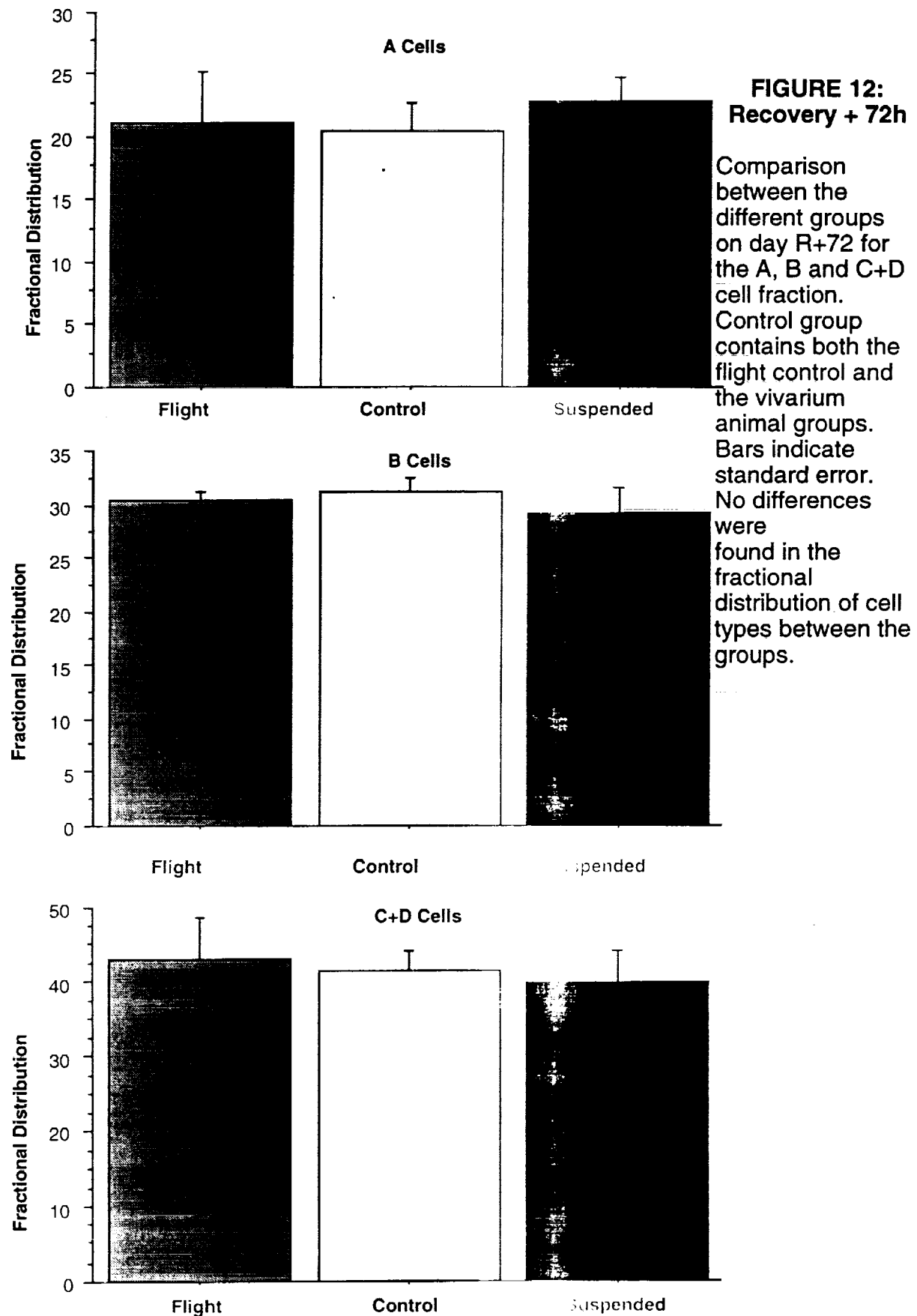
**FIGURE 10:  
Recovery + 0h**

Comparison between the different groups on day R+0 for the A, B and C+D cell fraction. Control group contains both the flight control and the vivarium animal groups. Bars indicate standard error. No differences were found in the fractional distribution of cell types between the groups.



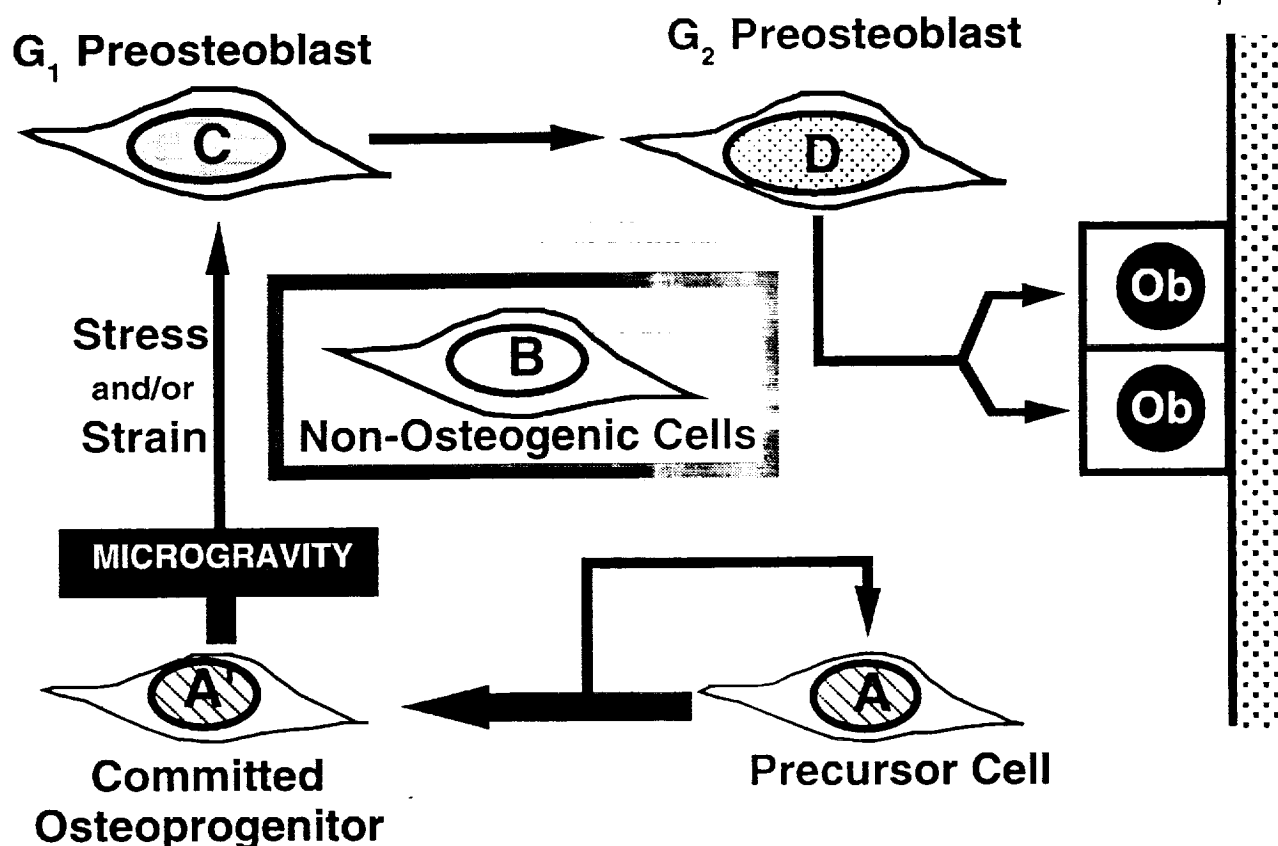
**FIGURE 11:  
Recovery + 24h**

Comparison between the different groups on day R+24 for the A, B and C+D cell fraction. Control group contains both the flight control and the vivarium animal groups. Bars indicate standard error. No differences were found in the fractional distribution of cell types between the groups.



## Summary of PARE.03 Flight Data from Periodontal Ligament and Mandibular Condyle

The influence of a ~9 day space flight and ~4, 24 and 72 hour recovery period at 1 g was assessed in fibroblast-like osteoblast precursor cells from rat maxillary molar periodontal ligament (PDL) and mandibular condyle. Data from PDL demonstrated statistically significant reduction in osteogenic precursor production immediately following return from flight. This reduction recovered rapidly and by 24 h post-flight, most cell populations were back to pre-flight normal levels. These data support the previously proposed model of osteogenic inhibition by exposure to microgravity (Fig. 13). No effect of exposure to microgravity was apparent on the cell kinetic populations of the mandibular condyle. These were rapidly growing animals whose growth rate was not influenced by spaceflight. We hypothesize that the genetic drive to grow was a much more powerful stimulus than the microgravity exposure in the growth site region of the mandibular condyle analyzed for these studies. An appropriate test of the hypothesis that in slow-growing or non-growing animals, mandibular condyle osteogenesis will be inhibited is needed. Experiments studying adult animals are much more appropriate for understanding the influence of microgravity on the current human populations living and working for short and long durations in space.



**Figure 13**

Cell kinetic model of osteoblast histogenesis. Microgravity blocks the stress/strain rate limiting step of preosteoblast production.

#### **IV. Publications**

Jennermann, C., Garetto, L.P., and Roberts, W.E. Bromodeoxyuridine and 3H-thymidine labeling of S-phase cells in rat mandibular condyle. ASGSB Bulletin 7:80, 1993.

Roberts, W.E., Garetto, L.P. and Brezniak, N. Bone physiology and metabolism of dental implants: Fundamental rationale of clinical practice. In: Contemporary Implant Dentistry - Diagnosis and Treatment, edited by C.E. Misch. St. Louis: Mosby, 1993, p.327-353.

Garetto, L.P., K. Jarrell, Stookey, K., Morey-Holton, E.M. and Roberts, W.E. Recovery of osteoblast histogenesis in rat periodontal ligament following a 9-day spaceflight (PARE.03). ASGSB Bulletin 8: 51, 1994.

Becker, R.F., Garetto, L.P., Potter, R.H. and Roberts, W.E. The effect of hypergravity (2g) on osteoblast precursor cells in rat periodontal ligament. ASGSB Bulletin 8: 88, 1994.

Martines, L.E., Garetto, L.P. and Roberts, W.E. Effect of hypergravity on mandibular condyle and osteoblast differentiation. J. Dental Research, 73SI: 174, 1994.

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Miller, R.L. and Garetto, L.P. Effects of estrogen repletion on osteoblast differentiation in ovariectomized rats. J. Dental Research, 73SI: 242, 1994.